

ABEL (J. J.)

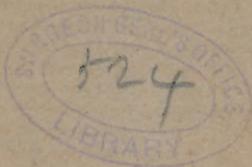
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A CONTRIBUTION TO OUR KNOWLEDGE OF ORGANIC
SULPHUR COMPOUNDS IN THE FIELD OF
ANIMAL CHEMISTRY.

BY JOHN J. ABEL, M. D., *Professor of Pharmacology.*

(From the Pharmacological Laboratory of the Johns Hopkins
University.)

presented by the author.



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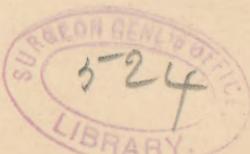
It is well known to workers in the field of animal chemistry that when freshly voided dog's urine is shaken up with milk of lime, or is made thoroughly alkaline with sodium or potassium hydrate, a peculiar, penetrating, offensive odor is developed; but beyond the merest passing references, nothing can be found in literature regarding the compound yielding this odor, and nothing seems to have been done toward determining its nature. Thus,² Böhm and Lange, discussing the applicability of Schlösing's method to the determination of ammonia in the dog's urine, remark that the addition to the urine of milk of lime gives rise to a peculiar, penetrating, garlicky odor, filling the whole bell-jar even after the lapse of 48-72 hours. V. Knieriem³ makes a similar reference, but no one has gone farther than to note the presence of this odor.

There are many substances known to chemistry which may be said to have a penetrating, offensive, stupefying, and in some cases, garlicky odor. Among these are the mercaptans, the organic sulphides, selenides and tellurides, the phosphines and the isocyanides, but in the present instance no help was

¹ The substance of a paper with the title: "On the occurrence of ethyl sulphide in the urine of the dog; on the behavior of ethyl sulphide dissolved in concentrated sulphuric acid toward oxidizing agents, and on certain reactions for the detection of alkyl sulphides," appearing in the December number of the *Zeitschr. f. physiol. Chemie.*

² *Archiv f. exp. Pathol. u. Pharmakol.*, Bd. 2, p. 368.

³ *Zeitschr. f. Biol.*, Bd. X, p. 269.



derived from the smell in identifying the body, as no one whose judgment was asked in the matter could state positively that the odor was like any other known to him.

I was obliged to direct my first efforts toward gaining some notion of the ultimate qualitative composition of the body. To this end I proceeded as follows: Air from an ordinary large glass gasometer was made to bubble through two liters of urine to which about 100 cc. of thick cream of lime had been added, and the whole thoroughly agitated for a few moments, and this air, laden with the odorous substance, was forced through an empty wash bottle, then through two Muencke's wash bottles made entirely of glass, each containing a 10 per cent. solution of hydrochloric acid, then through two similar wash bottles filled with a 40 per cent. solution of sodium hydrate, then through an empty bottle, and then through a piece of combustion tubing 60 centimeters long, filled in its middle third with asbestos fiber which had previously been heated piece by piece to a white heat in the flame of a Barthel's alcohol blast-lamp. Pure oxygen taken from an Elkan's cylinder and well washed by being made to pass through both acid and alkali, was forced into the tube containing the purified asbestos at the point where the air laden with the odoriferous body entered it.

From the tube filled with the asbestos the current of air was next passed into a Geissler potash bulb half filled with a 2 per cent. solution of the purest hydrate of sodium. With the apparatus arranged as described, the combustion tube containing the asbestos was kept at a red heat in a short four-burner furnace for five hours, the air and oxygen passing at about the rate that is usual in combustion analyses.

At the end of this time a portion of the sodium hydrate solution in the Geissler bulb was acidulated with hydrochloric acid and tested with a few drops of a solution of barium chloride, with the result that an immediate precipitation of barium sulphate occurred. It may here be noted that the air current after it had passed the heated tube turned a blue litmus strip red, whereas it had no action on litmus before being heated, thus demonstrating that the sulphur of our compound had been turned from a neutral into an acid combination. A blank experiment in which the urine was replaced

by distilled water was carried on for six hours, but in this case the sodium hydrate yielded no trace of sulphate. Our conclusion must therefore be that the volatile, odoriferous compound contains sulphur.

At this point two questions present themselves: First, can this sulphur compound be referred for its origin to the action of the hydrate of calcium upon one of the known so-called¹ "neutral" sulphur compounds of the urine, such as cystin and allied compounds, or such as hyposulphurous, sulphocyanic or mercapturic acids?

Second, does the air after passing through the system of wash bottles used in the combustion experiment, contain any other substances than this odoriferous compound?

The first question can be answered with considerable certainty by the method of exclusion. Cystin does not seem to be decomposed by treatment with milk of lime at room temperature. Baumann and Brenziger² have shown, however, that when ethyl cystein is heated with a free alkali, ethyl mercaptan is split off. M. v. Nencki³ was the first to demonstrate that the nauseating odor of the urine after asparagus has been eaten is due to methyl mercaptan, and also that methyl mercaptan is one of the products of the bacterial decomposition of proteids.⁴ Karplus⁵ has also found methyl mercaptan in the urine as the product of a special bacterium, and L. v. Nencki⁶ finds that it is always present among the gases of the large intestine. Because, then, of the proved occurrence of mercaptans in animal fluids, one cannot neglect making the proper tests for them where an offensive, not strictly definable odor is met with. But in the case under consideration the making of such tests will be seen to be unnecessary, for the very process of setting free our odoriferous compound with alkalies would have bound the mercaptans and prevented them from leaving the bottle, and an impassible barrier would also have been found in the wash bottles filled with sodium hydrate.

¹ Salkowski: Archiv f. path. Anat. u. Physiol., Bd. 58, p. 472.

² Zeitschr. f. physiolog. Chem., Bd. 16, p. 565.

³ Archiv f. exp. Pathol. u. Pharmakol., Bd. 28, pp. 206-209.

⁴ M. v. Nencki and N. Sieber: Monatsh. f. Chemie, Bd. 10, pp. 526-31.

⁵ Archiv f. pathol. Anat. u. Physiol., Bd. 131, pp. 210-222.

⁶ Sitzb. d. kais. Akad. in Wien, Mathem. Classe III, Abth. 98, pp. 437-8.

As for the other neutral sulphur compounds of the urine, the acids above mentioned, it may be remarked that the calcium and alkali salts of at least one of them, sulphocyanic acid, is stable and therefore could not come in question. The alkali salts of hyposulphurous acid are readily soluble and stable; the calcium salt is equally soluble but unstable.

Now, to exclude hyposulphurous acid as a possible source of the sulphur found in our combustion experiment, we have only to state that this experiment yielded the same result when fixed alkali instead of milk of lime was used to free the odoriferous substance. But even with the employment of calcium hydrate it seems hardly possible for sulphur dioxide to escape from a fluid containing such an excess of lime.

As to the mercapturic acids being a possible source of our sulphur compound, we have only to note Baumann's¹ discovery that when they are decomposed with alkalies mercaptans are split off, a fact that has been adopted into the methods of urinary analysis. But we have demonstrated that it would be impossible for a mercaptan to pass over into the combustion tube. A further proof that excludes both the mercaptans and also sulphureted hydrogen is seen in the fact that two strips of filter paper moistened with alkaline lead solution and placed, the one between the bottle of urine and the first wash bottle, and the other between the last wash bottle and the combustion tube, never showed the slightest change of color.

It is therefore fair to conclude that our sulphur compound is not a derivative of one of the known "neutral" sulphur compounds of the urine, but that it is split off by the milk of lime from a still unknown sulphur compound of the urine.

We now turn to the second question: Does the air after passing through the system of wash bottles used in the combustion experiment contain any other substance besides this odoriferous compound? If we bear in mind the contents of the series of wash bottles, it will be seen that no substance with acid or basic properties could have passed them; in other words, that only a chemically indifferent substance could have been found with the sulphur compound at the end of the series of bottles. This disposes not only of the mercaptans, but of

¹ Baumann : Zeitschr. f. physiolog. Ch., Bd. 8, p. 194.

all the various compounds spoken of in the beginning as having an odor similar to the compound in question, with the sole exception of the organic sulphides.¹

The phosphines, too, are excluded, for, being basic substances,² they would be held back by the hydrochloric acid. Then, too, the phosphines are very readily oxidized, and in small amounts could not be made to bubble up through so many wash bottles without being destroyed by this long contact with the air. A long series of experiments was, in fact, undertaken to determine whether or not a volatile phosphorus compound was present along with the sulphur compound before it was washed, but all with negative results.

It therefore seemed fair to assume that our sulphur compound was the only substance carried by the air through the series of wash bottles, and all the subsequent experiments made to establish its identity furnish additional support for this assumption.

SOLUTION OF THE COMPOUND IN CONCENTRATED SULPHURIC ACID, AND PROPERTIES OF THIS SOLUTION.

A series of experiments was next undertaken to determine how this sulphur compound could be collected in sufficient quantities for study and analysis. It was finally found that concentrated sulphuric acid completely absorbed the body and the resulting solution is without color and also without odor, unless the air current is allowed to bubble through the sulphuric acid for several days, when a faint oniony smell is perceptible. In concentrated sulphuric acid we have then a means of storing considerable quantities of this sulphur compound.

Chapman suction pumps were used to draw air through large flasks containing urine and milk of lime, then through two wash bottles containing a 10 per cent. solution of hydro-

¹ The selenides and tellurides are obviously out of the question as constant products of animal metabolism, but see a late interesting paper by F. Hofmeister in Arch. f. exp. Pathol. u. Pharmakol., Bd. 33, p. 198, on the ability of the organism to form the nauseating methyl telluride and selenide on the introduction of selenium and tellurium or the salts of their acids.

² With the exception of the primary phosphines.

chloric acid, then through two wash bottles filled with a 40 per cent. solution of sodium hydrate, then through a U tube 12 inches high and 1 inch in diameter filled with pieces of potassium hydrate, then through two similar U tubes filled with granular calcium chloride, and from this last tube the air laden with the sulphur compound passed through a Geissler bulb or through a small wash bottle containing concentrated sulphuric acid. The connections between the bottles were glass to glass held in place by the best black English tubing. Two such circuits were usually kept at work by the same suction pump, and the pump was operated day and night. After about 72 hours, in which time 15-20 liters of dog's urine had been exhausted of the sulphur compound, the Geissler bulb containing sulphuric acid was replaced by another. On diluting with water some of this concentrated sulphuric acid that has been charged with the body, or on neutralizing it with free alkalies, or on the addition of almost any metallic salt at hand, an intense odor like that of one of the organic sulphides pervades the room. These properties of dissolving in considerable quantities in sulphuric acid and of forming with it a nearly or quite odorless solution, and of being set free unchanged on dilution with water or on neutralization of the sulphuric acid, are possessed, so far as I know, by the sulphides¹ only among organic sulphur compounds. I have repeatedly dissolved 10 or 12 grams of ethyl sulphide in 100 cc. of concentrated sulphuric acid, having previously cooled both fluids, and found that the resulting solution had no odor whatever, but on adding to this solution half its weight of water, or, better still, of ice, the ethyl sulphide soon appeared floating on the top of the diluted sulphuric acid. Dimethyl sulphide and methyl ethyl sulphide were found to behave in the same way. Reference books on chemistry do not mention this property, and it was only after the completion of my experiments that I learned that this method is employed in refining crude Ohio petroleum, and that Mabery and Smith² had by its help

¹ In all probability, however, the analogous selenides and tellurides behave in the same way toward concentrated sulphuric acid.

² Americ. Chem. Journ., Vol. 13, p. 243, and Vol. 16, p. 38. R. H. Smith has also treated ethyl sulphide with an equal bulk of strong sulphuric acid diluted with much water and neutralized with barium

recovered alkyl sulphides from the "distillates of crude petroleum."

OXIDATION OF THE URINARY SULPHIDE.

The alkyl sulphides are readily oxidized to the fluid sulphonydes, and then further to the very stable crystalline sulphones. Thus, if ethyl sulphide, $(C_2H_5)_2S$, is oxidized with nitric acid, specific gravity 1.2, ethyl sulphonide, $(C_2H_5)_2SO$, is produced, a thick, unstable fluid, easily soluble in water. If, however, fuming nitric acid is used, then diethyl sulphone, $(C_2H_5)_2SO_2$, results, which crystallizes in large rhombic plates very soluble in water, melting at 70° and distilling at 248° without decomposition. The sulphonides have the property of being reducible with zinc and sulphuric acid to the original sulphide. The sulphones, however, are very stable substances, unaffected by treatment with zinc and sulphuric acid. It was hoped that oxidation of the concentrated sulphuric acid solution of the sulphide from the dog's urine with potassium permanganate would yield a sulphone, the composition and properties of which would determine which particular sulphide we were dealing with. Accordingly, about 100 cc. of a concentrated sulphuric acid solution containing the sulphur compound collected from about 25 liters of dog's urine was treated in the following manner: A beaker containing it was placed in pounded ice, and from time to time were added a few drops of a concentrated aqueous solution of potassium permanganate, also cooled to $0^\circ C$. When the permanganate ceased to be decolorized, a cold 4 per cent. solution of sulphuric acid was slowly added until the acid in the beaker was reduced in strength to about a 20 per cent. solution. The whole was then heated on the water-bath, while potassium permanganate was again added.

Under the influence of the heat an additional quantity of the permanganate was reduced, and the heating on the water-bath was kept up as long as any permanganate was decolorized.

carbonate for the purpose of forming barium-ethyl-hyposulphite, but makes no mention of the separation of the sulphide on dilution with water. Journ. of the Chem. Soc., 22 (1869), p. 302.

The slight excess of permanganate, when reduction no longer occurred, was removed by the addition of a little sodium formate.

The solution was then made alkaline with potassium hydrate, evaporated till crusts of potassium sulphate formed; the potassium sulphate filtered off, again concentrated, again filtered, and now evaporated to dryness. The dry residue was extracted with a little absolute alcohol, half of the alcohol evaporated on the water-bath and the rest allowed to evaporate spontaneously. A small quantity of a deliquescent compound remained. To remove all trace of potassium carbonate, the residue was extracted a third time with absolute alcohol, and the alcohol again evaporated. On the addition of dilute sulphuric acid to some of the deliquescent residue, the odor of acetic acid became very apparent. The characteristic odor of acetic ether was at once brought out on gently heating with the addition of concentrated sulphuric acid and alcohol, and the addition of ferric chloride to a neutral solution gave the characteristic blood red color of a solution of ferric acetate. These properties, viz., the deliquescence of the potassium salt, the odor of the free acetic acid and of its acetic ether, and the color of its ferric salt, leave no doubt of the occurrence of acetic acid as an oxidation product of the sulphur compound under examination. I was unprepared for this outcome, as I had hoped to secure a sulphone. On the supposition that a little of the sulphone might yet be mixed with the acetate, I acidulated its aqueous solution with sulphuric acid, drove away the acetic acid as far as possible on the water-bath, and again extracted the dry residue with alcohol, but only a trace of potassium acetate and sulphate was taken up. I now repeated the oxidation experiment twice over, making slight variations in the method, such as the employment of finely powdered potassium permanganate instead of an aqueous solution, and sodium instead of potassium hydrate, in the neutralization. I used also a little less than the necessary amount of permanganate, so as to avoid the after-use of sodium formate, but the final outcome was the same as before: an acetate again appeared.

A blank oxidation experiment was next undertaken in order to determine whether the reagents used contained any

thing oxidizable to acetic acid, but not a trace of acetate was found.

We may therefore safely conclude that our sulphur compound contains one or two ethyl groups. It is not easy to draw conclusions as to the presence of a methyl group in the sulphide, as such a group would have been oxidized to carbon dioxide and water. Carbon dioxide is, in fact, given off when the acid solution and permanganate are boiled, but this can be referred to the destructive oxidation of some of the sulphide, for it also takes place when synthetically prepared diethyl sulphide is treated in the same way.

OXIDATION OF SYNTHETICALLY PREPARED ETHYL SULPHIDE UNDER THE SAME CONDITIONS.

It was now in order to compare the behavior of synthetically prepared ethyl sulphide with our sulphide. Accordingly, a preliminary experiment was first made as follows: Five grams of ethyl sulphide, which distilled at 91.9°–92° C., were dissolved in 50 cc. of sulphuric acid, and oxidized by adding small quantities of powdered permanganate very gradually to the concentrated sulphuric acid kept in a freezing mixture, an hour and a half being consumed in adding 12 grams. When too much was added at one time, a flash of light would appear, showing that some of the sulphide was being completely oxidized, and the odor of ethyl sulphide also became apparent. 25 cc. of cold concentrated sulphuric acid were now stirred into the mixture, and from time to time small portions of a concentrated aqueous solution of permanganate were added together with about 20 cc. of water. A colorless solution of an oniony odor now resulted and this was slowly diluted with water up to a liter, permanganate still being added. The solution was then boiled for a short time and as the permanganate was still being reduced, more was added. When no more permanganate was reduced about 200 cc. of the fluid was distilled off. This distillate, which was plainly acid, was caught in a little strong potassium hydrate and subsequently enough more hydrate was added to give a neutral reaction, and it was then evaporated on the water-bath. There was obtained a small quantity of a deliquescent salt consisting of potassium carbonate and potassium acetate, which

gave the reactions that we have already mentioned as sufficient to identify acetic acid. In a similar experiment it was observed that when the distillation was undertaken before the oxidation was complete, that is, at a time when the permanganate was still being reduced, the distillate had a most disagreeable odor, reminding one both of onions and of acetic acid. Also when evaporated to dryness with an alkali, it gives all the reactions of a sulphite, viz., it reduces permanganate, it yields sulphureted hydrogen on reduction with zinc and sulphuric acid, it decolorizes an iodine starch solution, it gives a red color to a weak solution of sodium nitroprusside, and it gives off the peculiar stinging odor of sulphur dioxide on acidulation with sulphuric acid. In such a case, therefore, one must first oxidize the sulphurous acid with permanganate before undertaking the tests for acetic acid or the preparation of one of its salts. When boiling is resorted to in order to hasten the progress of the oxidation, it is found that much carbon dioxide is given off. It is evident that in the above experiments the greater part of the sulphide was oxidized to the end products, sulphuric anhydride, carbon dioxide and water.

As, to my knowledge, the alkyl sulphides have hitherto only been oxidized to sulphoxides and sulphones, and not as in the manner indicated above, to acetic and sulphuric acids, it seemed worth while to attempt the quantitative oxidation of ethyl sulphide to these latter products. Accordingly, 12 grams were dissolved in 100 cc. of concentrated sulphuric acid, the latter being cooled in a freezing mixture; 65 grams of finely powdered permanganate, somewhat less than the quantity theoretically necessary to oxidize the sulphide to acetic and sulphuric acid, were then dissolved in 200 cc. of cold sulphuric acid, and this solution was slowly added to the cold solution of the sulphide. The permanganate was at first entirely decolorized and none of the sulphide was liberated or destroyed, but toward the end of the operation, as more and more of the oily heptoxide of manganese from the bottom of the beaker came to be added, the black mixture began to foam up, flames now and then shot forth and much carbon dioxide was given off. A repetition of the experiment led to no better results. In both cases, however, water was added to the

black, agitated mass, and after diluting to about a liter, the odor of acetic acid became plainly perceptible. When diluted to several liters and distilled, a little of the distillate treated as before gave all the tests for acetic acid. A little silver acetate was also produced which crystallized out of water in long, shining needles, gave off fumes of acetic acid, deposited silver on gentle incineration, and also emitted the odor of ethyl acetate on treatment with concentrated sulphuric acid and alcohol.

On account of the fact, however, that by far the greater part of the sulphide had been destroyed, it was evident that it would be useless to attempt the estimation of the amount of acetic acid produced. It would seem, therefore, impracticable to oxidize ethyl sulphide in this way with the intention of securing a large output of acetic acid, but any one can convince himself by an off-hand experiment that acetic acid is one of the products of the oxidation of diethyl sulphide under the above circumstances. This is another point of agreement between the sulphide from dog's urine and ethyl sulphide.

What light this oxidation throws on the nature of the union existing between ethyl sulphide and concentrated sulphuric acid, and also whether thio-acetic acid may not be an intermediate product in the oxidation with permanganate, I cannot here discuss.

DOUBLE COMPOUND WITH MERCURIC CHLORIDE.

As the new sulphur compound from the dog's urine has so many points in common with ethyl sulphide, its behavior towards mercuric chloride was next examined.

Some of the concentrated sulphuric acid solution of the sulphide from the dog's urine was placed in an ice mixture and diluted with a cold 4 per cent. solution of sulphuric acid until the resultant liquid was equal in strength to about a 30 per cent. sulphuric acid solution. The odor found to arise from such large amounts of the diluted fluid was very strong, and not to be distinguished from the odor of ethyl sulphide dissolved in concentrated sulphuric acid and treated in the same way. Indeed, none of the workers in my laboratory could tell in any given case whether I was using the com-

pound obtained from dog's urine or that synthetically prepared. The diluted solution of the sulphide was now shaken out with ether and the separated ether was washed twice with distilled water; an alcoholic solution containing 1 gram of mercuric chloride was then added to the ether and the whole evaporated to about one-third on the water-bath, after which it was allowed to stand *in vacuo* over sulphuric acid. The residue, which smelled strongly of the sulphide, was well washed on a filter with water in order to dissolve away the excess of mercuric chloride. It was then dried on the filter over sulphuric acid, dissolved when dry in a little alcohol and allowed to crystallize. Some of the crystals, long, slender prisms mixed with some amorphous material were collected and their melting-point taken without further purification. This was found to be in one case about 145° C., and in another batch of crystals prepared in a very similar manner, 150° C. The noteworthy fact in both determinations was that the crystals melted to a black fluid, and after the capillary tubes had cooled, long slender prisms could be seen to stand out from the congealed drop.

I am personally convinced that this sulphide from the urine of the dog forms a double compound with mercuric chloride, although it must be admitted that the evidence, so far as its melting-point is concerned, does not furnish conclusive proof that this compound is $(C_2H_5)_2S \cdot HgCl_2$.

In support of this opinion that a double compound is formed, we may urge the odor of the compound, its insolubility in water, its solubility in alcohol, its behavior in the melting tube and its crystalline character. After standing over sulphuric acid *in vacuo*, no odor, or at least only a very faint odor, is perceptible; but exposed to the air for only a few moments, the odor of the sulphide becomes very marked. The mercuric chloride compound of ethyl sulphide behaves in the same manner.

It is exceedingly difficult to separate a small amount of this unstable double compound, say a few centigrams, from an excess of mercuric chloride; the various operations, such as the long and repeated washings with water, the necessary drying, etc., all involve so much loss of substance that sharp results cannot be obtained when there is only little material on hand.

MELTING-POINT OF THE DOUBLE COMPOUND,
 $(C_2H_5)_2S \cdot HgCl_2$.

The plan that was followed above in the attempt to secure and purify the double compound of ethyl sulphide from the dog's urine was based on previous experiments made in the same way with synthetically prepared ethyl sulphide. Five grams (boiling-point 91.9° C.) were dissolved in 50 cc. of concentrated sulphuric acid, and by following out the method described above, except that less of the theoretically required amount of mercuric chloride was used, the double compound crystallizing out of absolute alcohol in long, transparent, highly refracting prisms was obtained. This, recrystallized out of alcohol, washed with cold absolute alcohol and ether, and dried *in vacuo* over sulphuric acid and paraffine, began to melt at 118° C. and melted to a colorless fluid at 119° C. Subsequent recrystallizations out of ether caused no change in the melting-point. When the double compound is prepared by mixing alcoholic solutions of the sulphides and of mercuric chloride the melting-point is also 119°.

When some of the finely powdered crystals that show a melting-point of 119° are allowed to stand over sulphuric acid for two weeks, the melting-point is found to have risen to 131° C. Some of the unbroken crystals, however, that had stood for the same length of time over sulphuric acid melted at 120° C. When the temperature reached 180°–185° C. a rapid evolution of gas bubbles took place, but the liquid remained transparent and did not blacken.

Now Loir¹ gives 90° C. as the melting-point of $(C_2H_5)_2S \cdot HgCl_2$, crystallized out of ether. An observation made by me in the course of the above experiments may perhaps explain how Loir came to put the melting-point at 90° C. I prepared some of the ethyl sulphide mercuric chloride, recrystallized it out of absolute alcohol, washed it with absolute alcohol and ether, and exposed it for half an hour to an air current produced by a Bunsen suction pump. At the expiration of this time the melting-point was taken and it was found that the substance melted at 80° C., yielding a perfectly transparent, colorless liquid. After standing for twelve hours over sulphuric acid *in vacuo* the melting-point was found to have risen to 119° C.

¹ Ann. d. Chem. u. Pharm. 87, p. 370.

It may be remarked in passing that Blomstrand¹ finds one of Loir's melting-points, that of the platinum compound $2(C_2H_5)_2S \cdot PtCl_4$, 70° too low, it being in reality 178° instead of 108° as given by Loir.

BEHAVIOR OF THE SULPHIDE TOWARD SOLUTIONS OF
BROMINE AND IODINE.

It must be remembered that we are confined to solutions of the urinary sulphide in concentrated sulphuric acid for a study of its properties. The behavior of bromine and iodine toward these solutions is characteristic and in every respect like their behavior toward similar solutions of synthetically prepared ethyl sulphide. If a drop or two of a 2 per cent. solution of bromine in potassium bromide be added to a sulphuric acid solution of the urinary sulphide, or to an equally weak solution of ethyl sulphide, it will be observed that bromine is absorbed. The same thing is observed when bromine vapor is allowed to fall into a sulphuric acid solution that has previously been diluted with a few drops of water. If the sulphuric acid be poured off from the undissolved drop of bromine after having been thoroughly agitated with it, and then be diluted with water, it will be found that the sulphide odor no longer returns. If a piece of pure washed zinc and a little more concentrated sulphuric acid be added, the sulphide odor returns as the reduction proceeds. A solution of ethyl sulphide of about the same strength as that from the dog's urine behaves in the same way. But if a strong solution be made, the absorption of bromine is very evident, for now considerable bromine may be added before some of it remains undissolved. Such a concentrated solution of ethyl sulphide treated with bromine still smells somewhat of the sulphide after dilution with water, but if left to stand for a few days the odor disappears, and may then be caused to reappear on reduction with zinc and sulphuric acid. We are in all probability dealing here with the bromine addition compound $(C_2H_5)_2SBr_2$, which, as described by Rathke,² forms with water a colorless solution. Out of its aqueous solutions, iodine in potassium iodide precipitates an iodine addition product, $(C_2H_5)_2SI_2$, as a dark oily fluid.

¹ Jour. f. pract. Chem. (n. f.), Vol. 24, p. 190.

² Ann. d. Chem. u. Pharmac., Bd. 152, p. 214.

Far more striking is the behavior of iodine, the study of which has led to a reaction which may under certain circumstances serve to indicate the presence of an alkyl sulphide. On the addition of a few drops of a 6-10 per cent. solution of iodine in potassium iodide, or of a $\frac{1}{20}$ normal iodine solution, an immediate precipitation occurs. The sulphuric acid solution becomes a dark brown, turbid fluid in which a precipitate of infinite fineness is suspended. After standing over night a small quantity of a dark brown oil separates out in minute droplets and settles to the bottom. This is undoubtedly the addition product $(C_2H_5)_2SI_2$.¹

If the acid be poured off and water be added to this oily substance, the odor of a sulphide becomes at once apparent. The addition of a few drops of potassium hydrate immediately causes the oil droplets to dissolve, and brings out the sulphide odor in full strength. Iodine solutions also cause the dark cloudy precipitations in sulphuric acid solutions of the sulphides even when these are very much diluted with water, so that this reaction must be regarded as a very sensitive one.

Furthermore, a drop or two of the sulphide shaken up with much distilled water, say 60 cc., also gives a cloudy precipitate on the addition of a $\frac{1}{20}$ -normal iodine solution, and this precipitation occurs even when the aqueous solution has been allowed to stand for weeks, when we may be sure that the ethyl sulphide is really dissolved and not merely suspended. Out of these aqueous solutions of the sulphide to which iodine solutions have been added, the oily product referred to also settles on standing. This last reaction demonstrates very clearly that ethyl sulphide, contrary to the usual statements, is by no means insoluble in water. I daresay that its solubility in water is fully equal to that of ethyl mercaptan.

Methyl sulphide and methyl ethyl sulphide behave in almost the same way toward solutions of iodine. The oily compound that is precipitated from dilute solutions of methyl sulphide in sulphuric acid seems, however, to pass again into solution on standing.

If to a distillate of dog's urine that has been shaken with milk of lime or made strongly alkaline with a free alkali, a

¹ Rathke, *loc. cit.*

few drops of an $\frac{N}{20}$ -iodine solution be added, a cloudy precipitation, very like that seen under the same circumstances in aqueous solutions of ethyl sulphide, will be observed. In this instance, however, the reaction is of uncertain meaning, for Schiff¹ has shown that the distillate of the dog's urine contains a primary amine, and Abbott² has found that aqueous solutions of amines give cloudy precipitates on addition of $\frac{N}{20}$ -iodine solution.

BEHAVIOR OF ETHYL SULPHIDE TOWARDS NITROUS ACID.

While trying to establish the identity of the sulphide treated of in this paper, I observed that when a drop of an aqueous 5 per cent. solution of sodium nitrite was added to some of the sulphuric acid solution of the sulphide from the dog's urine, the latter at once took on a beautiful deep green color. A drop or two of Liebermann's³ nitroso sulphuric acid solution gives the same color and is preferable as a reagent to an aqueous solution of a nitrite, as if used in excess it does not so readily cause the disappearance of the green color. The color persists for some time, but disappears if the solution is left to stand over night, and when the nitrite is not added in excess the reaction will be found to be of great delicacy.

To get this reaction with the urinary sulphide in perfection it is best to conduct the well-dried air and sulphide as described on p. 6, with at least 5 or 6 liters of urine in the circuit, through a few cc. of concentrated sulphuric acid in a test tube an entire day. Special attention must be given to the drying of the air laden with the sulphide, for it is only when a completely dry current is passed into the concentrated sulphuric acid for the length of time named that a solution is obtained which will give at once the deep green color

¹ Zeitschr. f. physiol. Chemie, Bd. IV, p. 54.

² Private communication from Dr. A. C. Abbott, of the hygienic laboratory of the University of Pennsylvania, on the detection of amines in sewer air with $N/20$ -iodine solution, which induced me to study the behavior of aqueous solutions of ethyl sulphide toward iodine solutions.

³ Ber. d. deutsch. chem. Gesellsch., Bd. 20, p. 3231 b. In making up the solution of a nitrite in conc. sulph. acid I used sodium instead of potassium nitrite.

referred to, although solutions that have not remained so long in the circuit will also give a tinge of green, in which case, however, only a mere trace of the nitrite, such as adheres to a glass rod dipped into a solution of it, should be added. Now a drop or two of pure ethyl sulphide dissolved in a few cc. of concentrated sulphuric acid gives identically the same reaction on the addition of a drop or two of a nitrite solution or of nitrose sulphuric acid.

WHAT CHEMICAL CHANGES OCCUR IN THIS REACTION?

As long as the color persists, the sulphide can be liberated by the addition of small pieces of ice or by dilution with water, but after standing over night exposed to the action of an excess of the nitrose sulphuric acid, the now colorless solution no longer throws out the sulphide on the addition of ice. The reason for this is that the sulphide has been slowly oxidized by the nitrous acid to a sulphoxide, while the latter is reduced to nitric or nitrous oxide. That this oxidation has occurred is demonstrated by adding a few pieces of zinc and allowing the reduction to continue for some hours and then diluting with crushed ice, when the original sulphide will again make its appearance. No further demonstration is needed to prove that the sulphide has been oxidized to a sulphoxide in the above experiment. Since methyl sulphide and methyl ethyl sulphide also behave in the same way toward nitrose sulphuric acid, it is fair to conclude that the reaction holds for the series of sulphides of the general formula $(C_nH_{2n+1})_2S$.

It might be suspected that the color reaction just described is due to thiophene formed on dissolving the sulphide in concentrated sulphuric acid, in analogy with the pyrogenous synthesis of thiophene first demonstrated by Kekulé.¹ That we are not dealing with thiophene is, however, shown by the absence of that characteristic play of colors (green, blue to purple) that is always observed when a little nitrose sulphuric acid is added to a freshly prepared sulphuric acid solution of thiophene, and by the fact that a solution of thiophene in concentrated sulphuric acid soon fails to give Liebermann's reac-

¹ See V. Meyer: Ber. d. deutsch. chem. Gesell., Bd. 18, p. 217 a.

tion, in consequence of the rapid conversion of the thiophene into thiophene sulphonic acid. Then, too, that the green color described does not owe its origin to thiophene is proved by the fact that solutions of ethyl sulphide in concentrated sulphuric acid do not give the indophenine reaction, a reaction quite as delicate as Liebermann's reaction for thiophene.

The mercaptans, too, do not appear to give this reaction. As is well known, these sulphur compounds, when dissolved in concentrated sulphuric acid, are changed to the corresponding disulphides. Sulphuric acid solutions of ethyl mercaptan, the only one of the mercaptans that I have thus far prepared for comparison, become murky and take on a reddish yellow color on the addition of a few drops of Liebermann's solution. The sulphides of the series $C_nH_{2n}S$ also fail to give this reaction.¹ Ethylene sulphide and propylene sulphide, when dissolved in concentrated sulphuric acid in small amounts, yield slightly green solutions. On the addition of a few drops of nitrose sulphuric acid the green color instantly disappears, giving place in the propylene solution to a yellowish turbidity, while the ethylene solution remains colorless. Methylene sulphide gives a colorless solution with concentrated sulphuric acid, which undergoes no change on the addition of the nitrose sulphuric acid.

It may be mentioned in conclusion that the vapor of pure ethyl sulphide was subjected to a destructive oxidation by passing it mixed with moist oxygen over asbestos heated to redness, exactly as described in the combustion experiment with the urinary sulphide in the early part of this paper. Here, too, the air that escaped from the combustion tube was laden with acid vapors, and an examination of the weak sodium hydrate solution in the Geissler bulb showed that sulphuric acid was present. When the supply of oxygen was insufficient, the bulb also contained sulphurous acid.

HAS THE SULPHIDE AN INTESTINAL ORIGIN?

The fact that methyl mercaptan is found among the gases of the large intestine,² and that there exists between ethyl

¹ This point was not established in time for its appearance in the German version of this paper.

² L. v. Nencki: Sitzb. d. kais. Akad. in Wien, Mathem. Classe III, Abth. 98, 437-438.

mercaptan and ethyl sulphide a close relationship, suggests for the latter a possible intestinal origin, and that after absorption it unites with a compound that prevents its oxidation to end products and allows of its excretion in the urine. But an experiment in intestinal antisepsis¹ with calomel performed on a large well-nourished dog, showed after six days of abstention from all food except water and the administration of a total of 8 grams of calomel during the last three days, no appreciable diminution of the amount of the sulphide yielded to concentrated sulphuric acid. While this result is not absolutely conclusive because of the lack of quantitative methods for estimating the sulphide, and also because we cannot be certain that the bacterial activity in the intestines was completely suppressed, yet the evidence, so far as it goes, is against the bacterial origin of the sulphide. More conclusive is the negative outcome of all attempts to find the sulphide in the faeces of the dog by the use of the methods successfully applied to the urine.

The negative outcome of both experiments at least points to the probability that ethyl sulphide is a product of retrogressive metabolism. The urine, too, appears to contain decidedly more of the compound when the dogs are put on an exclusively meat diet than when fed on the mixed diet of refuse from the hospital kitchens.

So far as I have been able to discover, crude petroleum is the only other natural source besides the dog's urine, of the saturated alkyl sulphides. Now that we have methods for their detection, it is not unlikely that they will be found to be as widely distributed as are the mercaptans. I hope soon to be able to offer something definite as to the properties of the compound from which the sulphide is liberated in the dog's urine on treatment with alkalis, and also to be able to isolate the pure sulphide in sufficient amount for the determination of its boiling-point, etc. It is only after having accomplished the isolation of the compound with which the sulphide is united that we can say anything definite as to amounts in which the sulphide is excreted. Since, however, easily demonstrable quantities of sulphuric anhydride can be obtained by oxidizing its vapor, one is justified in the opinion that it is

¹ Baumann : Zeitschr. f. physiol. Chem., Bd. 10, 1886, S. 129.

present in equal or greater quantity than are such compounds as sulphocyanic or thiosulphuric acid. And it seems fair to conclude that it will be found on quantitative estimation to answer to the still unidentified sulphur compounds in the dog's urine.

RESUMÉ.

It will be seen that the difficulties encountered in the collection and study of the new compound were not few. The following points have, however, been clearly established:

1. When dog's urine is treated with alkalies, an odoriferous compound is liberated which contains sulphur and which is taken up with avidity by concentrated sulphuric acid and from which it is again liberated on dilution with water or on neutralization. The odor arising during the progress of the dilution or neutralization is not to be distinguished from that of ethyl sulphide, $(C_2H_5)_2S$. Ethyl sulphide is likewise absorbed by concentrated sulphuric acid with great avidity.

2. Oxidation of the urinary compound in the form of its solution in concentrated sulphuric acid yields sulphuric and acetic acids, thus demonstrating the presence in it of an ethyl group. Oxidation of ethyl sulphide under the same conditions yields the same products.

3. Mercuric chloride forms with the urinary sulphide a double compound which behaves, as far as could be determined, in regard to odor, solubility and crystallization, like the corresponding ethyl sulphide mercuric chloride, $(C_2H_5)_2S \cdot HgCl_2$.

4. Bromine and iodine behave toward its solutions in concentrated sulphuric acid in every way as toward similar solutions of ethyl sulphide.

5. A nitrite added to its solutions in concentrated sulphuric acid gives the same intense green color as with solutions of ethyl sulphide.

6. The organic sulphide thus shown to exist in dog's urine is ethyl sulphide, $(C_2H_5)_2S$. The mixed sulphide, methyl ethyl sulphide $(CH_3 \cdot C_2H_5)S$, might be thought to have an equal claim with ethyl sulphide as a urinary constituent, since its solutions in concentrated sulphuric acid behave in the same way toward bromine, iodine and nitrous acid, and since the products of its oxidation by the method described are the same, but pure methyl ethyl sulphide that has been several

times rectified is easily distinguishable from ethyl sulphide by its odor, which has an additional smell like that of rotten cabbages, not possessed by the latter.¹

PURELY CHEMICAL RESULTS.

The points of more especial chemical interest are:

1. The oxidation of ethyl sulphide to acetic and sulphuric acids.
2. Its great solubility in concentrated sulphuric acid, and the ease with which it can again be liberated from this solution even when dissolved in minute quantities.
3. Its oxidation to a sulphoxide by nitrous acid and its green color reaction with this reagent.
4. Its solubility in water and the ease with which its aqueous solutions can be detected with solutions of iodine in potassium iodide.
5. Also to be noted is the fact that the melting-point of ethyl sulphide mercuric chloride lies at 119° C., and not at 90° C. as stated by Loir and since his time in all reference books on chemistry.

¹ See J. Finckh (Ber. d. deutsch. chem. Gesellsch., 1894, No. 9, p. 1239), who finds that these organic sulphides lose their nauseating odor on being repeatedly heated to 290°-300° C. in a sealed tube with powdered copper.

